REMARKS:

This is in response to the Office Action mailed on January 7, 2009.

The Examiner has objected to claims 1 to 6 as being obvious having regard to Tarelli et al. in view of Boratynski. Applicant respectfully submits that the currently pending claims patentably distinguish over the cited references for the reasons that follow.

The present invention is a method of glycating a protein. The first step of the method is combining a quantity of a reducing sugar with the protein in an aqueous solution. The next step is lyophilizing the solution to produce a lyophilized sample (lyophilizate) in which the water has been removed. The glycated protein product is produced in the following steps: The lyophilizate is placed under vacuum. The next step is incubating the lyophilizate at elevated temperature while it is maintained under vacuum.

The Applicant's inventive method achieves glycation of proteins by the sealing of a lyophilized mixture of protein and reducing sugar (lyophilizate) under a vacuum in a vessel and incubating at elevated temperature. Surprisingly, only a single homogeneous glycation product is obtained via a ketoamine linkage (see figure 1).

Tarelli et al.(JOURNAL OF PHARMACEUTICAL & BIOMEDICAL ANALYSIS, vol. 12, no.11, 1994, pp. 1355-1361) disclose that lysine vasopressin (LVP), a small polypeptide, reacts with lactose in aqueous solution to give several condensation products, assumed to be Schiff bases, resulting from the reaction of its N-terminal amino group and the ε-amino group of lysine. They also state, but provide no data or evidence, that that the same condensation products appear to be produced when an aqueous solution of LVP and lactose is lyophilized at 0°C to remove water followed by incubation of the lyophilizate so formed at 37°C and atmospheric pressure in contact with air containing 65% relative humidity.

Boratynski et al. (GLYCONJUGATE JOURNAL, vol. 15, mo. 2, Feb 1998, pp. 131-138) disclose that protein-carbohydrate conjugates can be produced according to the following

procedure. The first step is the to dissolve protein and sugar in a 1:1 ratio(w/w) in aqueous solution. The second step is to form a dry lyophilizate by lyophilization of the protein sugar solution at 0°C to remove the water. The protein-carbohydrate conjugate, along with other products, is formed in the third step by heating the lyophilizate at atmospheric pressure in an air oven at 95-100°C. The final step is addition of water to solubilize the conjugate and separate it from insoluble material that is formed.

Applicant previously submitted that the cited references do not disclose the use of in vacuo glycation, as claimed by the applicant. Applicant respectfully maintains its submission that there is no teaching or suggestion in Brodsky, Boratynski or elsewhere in the prior art that glycation of proteins can advantageously be carried out under vacuum.

The Examiner infers that as skilled artisans Tarelli et al. were aware of "vacuum techniques for glycating proteins" based on Brodsky et al. (US 4,971,954). The Examiner cites page 12, lines 5 – 12 as follows: "*The solubilization of glycated tendon by CNBr .....fractions were lyophilized and weighed.*" and states that Brodsky et al. "*teach the glycation of collagen proteins from tendon under vacuum*". Applicant respectfully submits that the Examiner has mischaracterized the Brodsky reference in concluding that this reference teaches that proteins can be glycated under vacuum.

The above statement from Brodsky et al. cited by the Examiner is in section 6 entitled "Collagen Extraction" (page 11 line 43). The opening statement of this section by Brodsky et al. is:

" Collagen was extracted from tendon by acid, by pepsin treatment, and by CNBr digestion. About 15 mg (wet weight) of glycated tissue was finely chopped with a razor blade and extracted in 10 ml of 0.5 acetic acid 4°C."

Applicant submits that three points should be noted. Firstly, the tendon is already glycated prior to being dried (lyophilized) under vacuum. As a result, Brodsky et al. were not using this step to achieve glycation. In the passage quoted by the Examiner, Brodsky et al. use the term "glycated

tendon" and therefore they are using the vacuum to remove water from the extract and not to achieve the glycation. Brodsky et al. confirm this point in the statement cited by the Examiner where they state "and dried in vacuum over NaOH". Secondly, in the present invention, the glycation does not take place in the lyophilzation step used by the Applicant and also by Brodsky et al., but in the subsequent step of placing of the lyophilizate under vacuum and heating the sealed container containing the lyophilizate under vacuum. Thirdly, Brodsky et al. are forthright in stating that all their glycation reactions are carried out in the conventional manner in liquid solution: Commencing at page 3, line 55, they give a lengthy description of the solution conditions and parameters and conclude by stating: (page 6, line 8).

"The liquid reacting mixture may also contain any chemical which does not adversely affect the reaction ...."

Nowhere in the statement from Brodsky et al. quoted by the Examiner or elsewhere do Brodsky et al. teach or suggest that a vacuum can be employed to promote glycation. In fact, Brodsky et al. teach away from the present invention in advocating the use of increased pressure, not decreased pressure or vacuum as evidenced by their statement at page 3, line 55 as follows:

"Thus the process of invention contemplates a range from about room temperature, e.g. about 20°C to 50°C. Likewise the speed of the reaction can be promoted by varying (e.g. increasing) the pressure from atmospheric to above atmospheric."

It is therefore respectfully submitted that based on Brodsky, it could not have been obvious to Boratynski et al. or Tarelli et al. or any other skilled artisan that incubating a lyophilized mixture of protein and reducing sugar under vacuum, i.e. at greatly reduced pressure, would yield glycated protein. Lyophilzation at 0°C (freeze-drying) is a long established procedure for removing water from biological materials. In both of these cases (as well as Brodsky et al.), lyophilization at 0°C under vacuum is used simply to remove water not to achieve glycation. The in vacuo glycation procedure of the present invention also has a lyophilization step at 0°C for the purpose of removing water. This step is not for achieving glycation. The inventive step of the

present invention is not that glycation is achieved in this lyophilization step, but that the glycation is achieved by subsequently heating this lyophilizate sealed in a vessel under vacuum.

Similarly, the cited references do not teach or suggest that a vacuum can be used to promote glycation of a lyophilizate. Further evidence that the use of a vacuum for promoting glycation was not obvious to Tarelli et al. is in their description of their glycation procedure under the section entitled "Experimental". It is stated at p. 1356, line 19 of Tarelli et al. as follows:

"Trial preparations of LVP in ampoules were prepared by lyophilizing aliquots (1ml) of a solution of LVP (20 μg ml^{-1}) and lactose (5 mg ml^{-1}) in 50 mM citrate or 50 mM acetate buffer (pH 4.5) according to published methods..... For isolation of products for structural purposes sodium cyanoborohydride was added to a reaction mixture containing 1 mg of LVP in 1 ml of 100 mM phosphate (pH 8). Incubation was continued at 37°C for a further 24 h and acetic acid (50 μl) was then added. The solution was then fractionated as described below."

From this passage, it is clear that the lyophilization procedure at 0°C used by Tarelli et al. was carried out simply to remove water, and when they wanted to produce stable glycation products, they did not use the lyophilizate but performed the reaction in aqueous solution using cyanoborohydride (see above statement) to stabilize chemically the product. The reason Tarelli et al. use cyanoborohdride is that they believe their glycated product is formed via an unstable Schiff base (p. 1360, line 20). Reaction with cyanoborohydride yields a reductively aminated product, a chemically different linkage than the ketoamine produced by the in vacuo glycation procedure of the present invention. This procedure used by Tarelli et al. is the conventional glycation procedure and the current state of the art for producing glycated proteins. This passage clearly demonstrates that it was not obvious to Tarelli et al. that stable glycated protein via ketoamine could have been produced by incubation of a lyophilizate under vacuum at elevated temperature, thus eliminating the need to add cyanoborohdydride in an aqueous glycation procedure.

In the case of Boratynski et al., they state quite clearly that their procedure does not use a vacuum to achieve glycation. They state under Materials and Methods at page 131 that *"Dry lyophilizate was then placed in an air oven at 95-125°C for 20-40 min with the tube left open."* It should be noted that, prior to the heating step, they also carry out a lyophilzation step at 0°C with protein and reducing sugar but do not state or claim that this produces any glycated protein. They provide evidence that carbohydrate becomes attached covalently to the protein after heating the lyophilizate in air at atmospheric pressure, but do not provide any evidence as to the chemical nature of the covalent linkage. Again, nowhere do Boratynski et al. state, infer or imply that it was obvious to them that the glycation could have been achieved with the formation of a ketoamine covalent linkage by subjecting the lyophilizate to a vacuum and elevated temperature. A subsequent publication (Yeboah, F.K., Inteaz, A., and Yaylayan, "Glycation of Bovine Serum Albumin" J. AGRIC. FOOD CHEM. vol 7, 1999, pp. 3164-3172) using the same dry lyophilizate procedure as Boratynski et al. state the following (p. 3167) concerning the products produced from a dry lyophilizate of reducing sugar and protein heated in air:

"*When molecular oxygen is present at the initial stages of the Maillard reaction, however, it catalyzes the oxidative reactions that lead to the formation of reactive dicarbonyls, free radicals and fragmentations*".

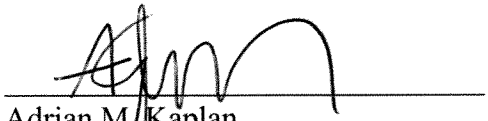
Thus the dry glycation reaction of a lyophilizate containing carbohydrate and protein reported by Boratynski et al. leads to a wide variety of products, soluble and insoluble, in addition to the glycated protein product that they claim is formed. The essential point is most of the products of their dry glycation reaction in air are different than the solitary and soluble well defined glycated ketoamine product (Figure 1 of patent application) formed by the in vacuo glycation procedure of the present invention.

In summary, the cited references demonstrate that it is not obvious to one skilled in the art that subjecting a lyophilizate of a reducing sugar and a protein to a vacuum and heating the lyophilized sample under vacuum would result in a single homogeneous glycation product with the protein by the formation of a covalent ketoamine linkage.

Favourable consideration and allowance of this application are respectfully requested.

Executed at Toronto, Ontario, Canada on March 6, 2009.

Harvey KAPLAN et al.



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